Review Article

Genetic Diversity, Zoonotic Risk and “One Health” Initiative of Bovine Brucellosis

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INTRODUCTION

Bovine brucellosis is one of the most important bacterial diseases affecting cattle (Corbel, 1997). The causative agents of this disease is Brucella abortus and with an emerging importance of B. melitensis are zoonotic pathogens that can cause a severe disease in humans, therefore highlighting the importance of its control (OIE, 2009). A wide range of animals is affected by the Brucellosis infection for example buffaloes, goats, sheep, camels, pigs and reindeers etc. other mammals are also affected but less frequently as compared to aforesaid cattle (Charters, 1980). Brucellosis is characterized by abortion, with excretion of the organisms in uterine discharge and in milk. Major economic losses result from abortion, loss of calves, and reduced milk yield in females and infertility in males and it can be diagnosed on the basis of abortion and discharged contents in milk and as well as uterine discharge (WHO, 1971). Brucellosis is a zoonotic infection and a solemn jeopardy to public health (Lapaque et al., 2005). Bovine brucellosis is occurring worldwide except where eradication program worked (Seleem et al., 2010). Entry sites for Brucella are all those sites which are in continuous directly exposed to the different pathogens. Although Brucella may enter the body by either route but major routes of entry are lungs, digestive tract, skin and mucosal layers etc. inspite of these routes Brucella may cause local infections by entering through blood and lymph. By entering through these routes it infects the tissue and causes local tissue damage (Lapaque et al., 2005).

Testing of livestock for brucellosis is done by culture and serology or by testing milk samples (Nielsen, 2002). The main serological test used for diagnosis of brucellosis is the Rose Bengal Plate agglutination Test (RBPT), which has very high (>99%) sensitivity but low specificity (Barroso et al., 2002). As a result, the positive predictive value of this test is low and a positive result is required to be confirmed by some other more specific test like serum agglutination test (SAT) and ELISA (Memish et al., 2002). With the advancement of molecular techniques it is now described that six species of Brucella contain almost > 90 % homology with each other. (Whatmore, 2009).

TAXONOMY OF THE BRUCELLA

Classification of genus Brucella has passed through different phases. Until 1985 the genus Brucella was classified into six species. But later on this classification was aborted and all the species were placed under one species. (Verger et al., 1985) But
again in 2003 the committee on Brucella taxonomy agreed to
reinstate the classification of 1985 and divided the Brucella into
six species. (Oysterman and Moriyon, 2004) In 2007 Brucella ceti
and Brucella pinnipediae (infecting preferentially cetaceans and
pinnipeds, respectively) were recognized as new species (Foster et al., 2007) In 2008, another new species of Brucella was
first isolated in the common vole (Microtus arvalis) it was
recognized as Brucella microti. (Scholz et al., 2008b) recently
Brucella inopinata was isolated from a breast implant infection in
an elderly woman with clinical signs of brucellosis. (Scholz et al., 2010) Recent reports describes isolation and
characterization of strains from marine mammals which are
known as Brucellae however characteristics are not similar with
already recognized Brucella species. (Clavera et al., 1998,
Cloeckaert et al., 2001, Jahans et al., 1997) According to current
taxonomic classification there are 10 species of the Brucella
genus in total which are recognized.

INVADING THE IMMUNE SYSTEM
Brucella is facultative intracellular organism which survives
within the host cell and bypasses the host normal immune
system (Ko’ hler et al., 2002, 2003; Baldwin and Goenka, 2006)
Brucella species at first infect the phagocytes and in these
phagocytic cells it can resist many stresses. And eventually
reach its replication site i.e. placental trophoblasts and here it
reuplicates extensively. (Kohler et al., 2002). It modulates
different immune cell functions. Such as in dendritic cells it
interferes with their antigen processing and also interferes in
their maturation. (Roop et al., 2009). It can also prolong the
survival rate and duration of immune cells, for example in
macrophages it prevents apoptosis and long term survival in the
reticuloendothelial system. (Gorvel and Moreno, 2002) After
bypassing the immune system Brucella reaches its replication
site and it focus on its replication. During pregnancy uterus is
the area which is immune privileged area. Here once Brucella
reaches it extensively replicates and causes abortion ultimately.
(Neta et al., 2010)

GENETIC DIVERSITY OF BRUCELLA
Different genetic combinations present in a genetic pool is
called as genetic biodiversity. Due to the importance of genetic
diversity studies most of the researchers are now focused not
only on the identification of the new markers but developing
and designing suitable techniques for the discrimination
between the genus currently under discussion to facilitate the
government bodies. Which in turn devise and plan for
epidemiological surveys and also for control and eradication
programs against this disease in their respective countries. It is
evident from aforementioned discussion that Brucella although
comprises of different species but these species have similarity
in their genomic make up.

It is evident that regardless of pathogenicity of the
different species of the Brucella, the genus has genetic
similarities with in species. (Whatmore, 2009) There is high
level of nucleotide similarities between Brucella species, but vary
in host tropism and pathogenicity. Now a day’s techniques are
being developed to assess the genetic biodiversity of Brucella.
(Vizcarrondo et al., 2000; Moreno et al., 2002; Bricker, 2002) It is
clear with the help of molecular techniques that there is high
degree of identity between the DNA of classical Brucella species.
On the bases of DNA-DNA hybridization techniques, More than 90 percent similarities are present among all species of
Brucella. (Hoyer and McCullough, 1968a, 1968b; Verger et al.,
1985).

POPOPULATION GENETICS OF BRUCELLA
There are different tools which are used to study genetic
diversity. One of the techniques used for such type of study is
Multi Locus Enzyme Electrophoresis (MLEE) technique
(Whatmore et al., 2007). It has been set as a gold standard for
population genetic studies and molecular epidemiological
studies of pathogenic bacteria. It is important to measures the
genetic diversity of the bacterial pathogen, the virulence factors
and susceptibility of the microbes towards antibiotics can be a
helpful tool for epidemiological studies. There is little evidence
that bacterial population studies can be applied to Brucella.

By using multilocus enzyme electrophoresis (MLEE) techniques
population genetics has been studied for Brucella. (Boerlin, 1997)
Some of the assays are suitable for gene level study of
Brucella. e.Pulsed-field gel electrophoresis, Insertion sequence
based typing, PCR typing, Restriction fraction length
polymorphism based approaches, Shift to ‘genome-based’
typing approaches, Tandem repeat based typing, Multilocus
sequencing and SNP typing.

COMPARISON OF GENOMICS
The availability of gene sequencing methods and techniques are
helpful to understand about the pathogenicity and biological
changes in the group under comparative study. In other words
it can be easy to compare genome as well as other features of
group members by the use of advanced research techniques. In
2002 B. melitensis 16 M genome was sequenced successfully.
Following this genome sequence was successfully done for
other strain of Brucella i.e. B. Suis 1330. (DeVecchio et al., 2002;
Paulsen et al., 2002). After comparison of genome of these two
strains it was found that their genomic make up is 98-100%
identical. After a gap of almost three years third strain of
Brucell’sa genome was sequenced. i.e. B. abortus genome
sequence was published in 2005. (Halling et al., 2005)

MULTILOCUS ENZYME ELECTROPHORESIS (MLEE)
It is revealed that all three genomes are very similar with each
other. These genomic studies helped in understanding the
pathogenicity. (Halling et al., 2005; Chain et al., 2003) An
extensive study was done on genomic similarity and
 dissimilarity of these three strains fully sequenced genomes.
After Examination of 2308 sequences it was found more than
hundred base pair are unique to B.melitensis and B suis. But
these unique base pair sequences are also found in B.
abortus.Later this was confirmed that B.abortus is most closely
related to B.melitensis. (Chain et al., 2005) Recently a full
sequence of vaccine strain of B.abortus S19 was published.
(Crasta et al., 2008) Multilocus enzyme electrophoresis
(MLEE) is standard method to assess the genetic diversity of
the bacterial genome. If genetic distance is larger than 0.5 than
recognize as new species. MLEE of 99 isolate of Brucella revealed
that there is very limited genetic diversity in this group.
(Gandara et al., 2001)

ZOONOTIC RISK
Brucellosis is the most important zoonotic disease worldwide
and it is responsible for huge economic losses affecting
livestock and human population. In most of the countries this
disease is endemic. (Godfroid et al., 2005) Control of this
disease is depends upon the rapid detection method which can
also applied in field. DNA based detection methods are now
developed to diagnose this disease. After the release of full
genome of Brucella it is now easy to understand the virulence
and pathogenesis of this disease.
TRANSMISSION
There are many factors which are responsible for transmission of the disease in livestock as well as in humans. And it is varies with geographically conditions, climate, age, sex and species. (Gul and Khan, 2007). In a study conducted by Abubakar et al. (2010) showed that incidence of the disease is increased with age and also increased in sexually matured animals. Brucellosis is transmitted in human by different means e.g direct animal contact, inhalation, consumption of unpasteurized milk and other dairy product and undercooked meat products. (Malik GM., 1997) Brucella can survive for long period of time in dung, water, dust, soil, aborted fetuses, meat and dairy products. And it is occupational risk for human, veterinarians and other related personals because of very low infectious dose. (Smits and Cutler, 2004) In female animals, it can reside in udder and secrete in milk and in male animals epididymitis and orchitis can lead to temporary or permanent infertility. The increase in travel from endemic to non-endemic area can increase the importance and eradication of this disease. (Corbel, 2006)

Worldwide more than 300,000 humans are affected with brucellosis. (Pappas et al., 2006)

ZOONOTIC IMPACT
Five out of nine species of Brucella can infect humans in which most pathogenic for human is B. melitensis. And followed in descending order by B. suis, B. abortus and B. canis (Acha et al., 2003). In last few years the zoonotic characteristics of the marine Brucella (B. ceti) has also been reported. (Brew et al., 1999; McDonald et al., 2006; Sohn et al., 2003). B. melitensis, B. suis and B. abortus are listed as potential bio-weapons by the Centers for Disease Control and Prevention because Brucella species are highly infectious in nature and can be easily aerosolized. Transmission typically occurs directly with skin lesion, inhalation of aerosols and consuming contaminated or unpasteurized dairy and milk products. (Young, 1998; Christopher et al., 2010). Due to resemblance of Brucella symptoms with influenza it is difficult to detect the outbreak. (Chain et al., 2003). Studies in the sub Saharan Africa suggest that cattle are the main source of the Brucella spp. And the Brucella abortus infected cattle are the main source of the causing disease in humans in sub-Saharan Africa. (McDermott and Arimi, 2002).

WORLDWIDE DISTRIBUTION AND ECONOMIC IMPACT
Worldwide human brucellosis prevalence has been studied. The high risk countries are south and Central America, Eastern Europe, Asia, Africa, Mediterranean Basin, the incidence of disease in the Eastern Mediterranean Region ranges from 1 per 100,000 to 20 per 100,000 populations. Brucellosis is endemic in Saudi Arabia, where the national sero-prevalence is 15% (Memish, 2001). The geographical distribution is constantly changes with new emerging and re-emerging centers. Brucellosis causes more than 500,000 human cases worldwide. This disease has a very limited geographical distribution but still have a notable status in the western Asia, Latin America and some part of Africa. (Seleem et al., 2010).
The countries like UK, Sweden, Netherlands, Canada, Cyprus, Denmark, Finland, New Zealand and Norway. The Eastern and northern Asia, Central Asia and central South America are still not free from brucellosis. In some countries the B. melitensis has never been reported. According to (Robinson, 2003), there are no reliable reports that B. melitensis is eradicated from small ruminants from any country. Due to some socioeconomic, sanitary and political issues brucellosis is still more prevalent in some part of the world. Even made advances in surveillance techniques. (Pappas et al., 2006) The disease cause huge economic losses in animal production system in the form of abortion, reduced milk yield and delayed conception and in public health in the form of cost of treatment and productivity loss. Annually 600 Million US dollar losses are due to brucellosis in Latin America. The US national brucellosis eradication program, while costing $3.5 billion between 1994 and 1997, the cost of reduced milk production and abortion in 1992 alone was $400 million (Acha et al., 2003; Sriranganathan et al., 2009).

MEASURES FOR CONTROL: ESPECIALLY IN DEVELOPING COUNTRIES
Brucellosis is highly infectious and contagious with rapid inter and intra herd spreading (Ahmad, 2005). The main important objective to control of this disease is reducing the impact of this disease on animal health and especially human concerns. An effective control measures include the surveillance of the infected herds with cost effective and more specific diagnostic tests (Abubakar et al., 2012). Separation of the infected herd from healthy ones and eradicate the reservoirs to protect the susceptible population in that area. Vaccination of the domestic animals and wildlife reduced the risk for human health.

Alexander et al., (2012) reported that the areas where buffaloes are present it is more likely that these species are may be the important in the transmission of the disease. And it is recommended that wild life species are also included in the surveillance studies. According to (Saleem M.N et al 2009) said that cost effective control measures for brucellosis is known but problem is lack of funding and awareness in respective authorities.

It is difficult to implement control strategies in developing countries considerable efforts have been required for the infrastructure and for awareness campaigns about Brucella risks. Providence Laboratory facilities and trained personals for collection and testing of samples for regular surveillance activities. So these are the challenges which are facing by the developing countries. By controlling disease in animals may reduce the risks in humans considerably. And its happen in developed countries where bovine brucellosis is controlled or eradicated the human risk becomes low. So for the control of the disease there should be strong support by the concerning government. The farmers, governments, milk industry and consumers must work together for the control of this disease.

Three types of control measures are described by the Abubakar et al., (2012) which are first: to eliminate the reservoir by proper quarantine and hygiene measures. Second: reduced or breaking the connection between reservoir and susceptible population. Third: by immunization with quality vaccines.

ONE HEALTH INITIATIVE AND BRUCELLOSIS
One health initiatives although is very successful for emerging zoonotic diseases but for brucellosis there is lack of conceptual frame work for the control of brucellosis. In developing countries there is no one health surveillance and control system for zoonotic disease. By implementing these initiatives for the control of the disease ultimately there are benefits for Public health and concerning societies. (Zinsstag et al., 2007)

Coker et al., (2011) said and it is obviously true that changes are occurs in the livestock sector and due to re-emerging of zoonotic diseases a new one health research and policies has to be defined. The most important thing is the correct diagnosis for the disease both in Humans and Livestock due to zoonotic pathogen. And assure that what type of species is involved in human and in livestock. This approach leads to the proper planning of the surveillance and control of the disease under one health initiatives. According to Jones et al.,
(2008), almost two third of the human pathogens are zoonotic and are of great concern because of causing deadly disease in humans. So it is important to have a global surveillance and control system for the emerging zoonotic diseases like brucellosis.

In trading live animals the OIE emphasizing on avoid transmission of the diseases. So it is important to have a worldwide standardized detecting system. The OIE also prescribed tests which are implicated in the field conditions and these are also appropriate in the developing countries. But the important thing is that rather emphasizing on detection systems it is important to detect the reservoir animals in the population from where disease occurs (Godfroid et al. 2012).

“One Health” initiative is only successful when all related professionals work together like veterinarians, medical personal, and wild life professionals. It will play a major role in the control of zoonotic diseases like brucellosis.

CONCLUSION

Brucella is a zoonotic pathogen which causes heavy losses in livestock as well as in public health. Developed world eradicate this disease by Test and Slaughtered method but this method is not suitable for developing countries, so to addressed these challenges it is important to control the disease in Livestock ultimately reduced risk for public health and obviously in livestock also. Developing countries or countries where this disease is endemic must be seriously planned and make strategies for the control and eradication of this disease. Now a day “One Health” Concept is being introduced which is very helpful in combating different zoonotic disease. After the discovery of Brucella Genome it is now easy to understand the virulence of this bacterium and how it causes the disease. And it may helpful in new diagnostic techniques for brucellosis and host specificity determination in future. New virulence factors are also recognized with the help of genetic techniques. After the above discussion it is recommended that “One Health” initiative should be started in developing countries which are in process to combat with this disease and also for other endemic zoonotic diseases. So in the “One Health” umbrella one should know his responsibilities and make strategies for control and eradication if zoonotic diseases that is suitable for each country and all professional work together for the well-being of humanity.

REFERENCES


